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RESPIRATORY ADAPTATIONS OF THREE SPECIES OF *UPOGEBIA* (THALASSINIDEA, CRUSTACEA) WITH SPECIAL REFERENCE TO LOW TIDE PERIODS

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Abstract

At simulated low tide, *Upogebia africana, U. affinis*, and *U. capensis* moved to the air-water interfaces of their burrows and took up positions in which the cephalothorax was out of the water. The posterior end of the gill chamber dipped into the water and continuing scaphognathite beat pulled water up through the gill chamber. This behavior was induced in *U. africana* by exposure to low oxygen tension (pO_2) water. The median survival time of *U. africana* was 18 h in anoxic conditions and 72 h in air. *U. africana* has a low metabolic rate that may be an adaptation to hypoxic conditions. The blood pO_2 decreased rapidly when the shrimps were exposed to hypoxia and reached an equilibrium level after about 5 h. The pO_2 of blood of *U. africana* in air was approximately half that of shrimps in normoxic water.

Introduction

Thalassinid shrimps occupy burrows in the soft substrates typical of many intertidal and shallow subtidal areas throughout the world (Macginitie 1930, 1934). These substrates frequently are hypoxic. In addition, those species living in intertidal areas cannot irrigate their burrows each day during periods of low tide. A number of behavioral and physiological respiratory adaptations to these hypoxic habitats have been described. Such adaptations in species of *Callianassa* include reduced metabolism at low oxygen tensions (Torres *et al.*, 1977), high affinity hemocyanin (Miller and Van Holde, 1974), tolerance to anoxia (Thompson and Pritchard, 1969), and burrow ventilation behavior related to oxygen tension (Farley and Case, 1968). Less information is available on respiratory adaptations of species of *Upogebia*, but they appear to be similar to those of *Callianassa*. Thompson and Pritchard (1969), for example, report that *Upogebia pugettensis* reduces its metabolic rates in low oxygen tensions and tolerates considerable anoxia.

The present study describes behavior under low tide conditions in burrows in three species of *Upogebia: U. africana*, a common inhabitant of intertidal mud banks in estuaries in southern Africa; *U. affinis*, which occupies a similar habitat on the Atlantic coast of North America (Pearse, 1945); and *U. capensis*, a marine species found on the southern and western coast of southern Africa, more commonly subtidally than intertidally (Barnard, 1950). Metabolic rates, tolerance to anoxia, and oxygen tension of the blood under hypoxia also were measured in *U. africana*.

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Abbreviations: Standard deviation, SD; standard error, SE; oxygen tension, pO_2; blood oxygen tension, p_iO_2; oxygen tension in surrounding water, p_eO_2.

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Materials and Methods

Source of material

All *Upogebia africana* specimens used in this study were collected from mud banks in the Kowie estuary at Port Alfred, South Africa. *U. capensis* specimens were obtained from the rocky shore around Port Alfred. Observations were made at Rhodes University, Grahamstown. *U. affinis* specimens were collected from intertidal mud flats at Beaufort, North Carolina, and behavioral observations made at Duke University.

Behavior at low tide

Observations were made on 10 specimens of *U. africana* kept in a glass-sided aquarium containing a 150-mm-deep layer of estuarine mud into which shrimp burrowed. Some sections of their burrows adjoined the sides of the aquarium, enabling observations of their behavior. Low tide was simulated by withdrawing all the overlying water for 6 h. Observations were made on 10 specimens of each species kept individually in U-shaped glass "burrows" with an internal diameter of 10 mm and a 20-mm-diameter enlargment at the base, where the shrimp could turn. The "burrows," with vertical height 300 mm, were filled to within 50 mm of the top with seawater (34‰). A 10–15 mm carapace–length shrimp was introduced and its behavior observed for 4–6 h. The oxygen tension of water around *U. africana* during low tide in glass burrows was determined from water samples withdrawn into a syringe and injected into a Radiometer BMS3 analyzer. The water bath of the analyzer was maintained at 20°C, the water temperature in the simulated burrows.

Behavioral response to low oxygen tensions

The effect of low oxygen tensions on *U. africana* behavior was determined on shrimp kept individually in a flowing-water system involving a U-shaped "burrow" as described above, except that 50 mm from its upper ends, the burrow was fitted with small side arms through which water (34‰, 18°C) flowed at about 3 $l \cdot h^{-1}$ from a supply reservoir. The oxygen tension of water in the system could be lowered by bubbling nitrogen through the water in the supply reservoir. An oxygen electrode was used to measure continuously the oxygen tension of water passing through the "burrow." A 10–15 mm carapace-length shrimp (1.5–2 g) was put into the burrow and allowed to settle down for 1 h at a pO_2 of 140–148 mm Hg. The oxygen tension was then slowly reduced to about 5 mm Hg over approximately 1 h. The pO_2 was then gradually increased by aerating water in the reservoir. The shrimp's behavior was noted at various oxygen tensions. The procedure was repeated on 10 animals. Control experiments were carried out using shrimps kept in the system in aerated seawater.

Tolerance to anoxia

Tolerance of *U. africana* to anoxia was determined in circular glass dishes 250 mm in diameter containing about 5 l (depth approximately 100 mm) of seawater (34‰, 18°C). Nitrogen was bubbled through the water by means of inlet and outlet tubes in the glass lids. The outlet tube was fitted with a water trap. When the oxygen tension of samples of the water was below 0.5 mm Hg, 10 shrimps were put into each of four dishes. A fifth dish containing aerated water and 10 shrimps

was used as a control. The number of shrimp alive was counted at irregular intervals for 24 h. Oxygen tension in the experimental dishes remained below 0.5 mm Hg. Water was not replaced during the experiment.

Survival in air

Two batches each of 20 specimens of *U. africana* were put into covered 250-mm-diameter glass dishes. The dishes contained a 10-mm-deep layer of freshwater, but a perforated plastic floor prevented the shrimp from contacting the water. Water-saturated air was pumped through the dishes at 400 $ml \cdot h^{-1}$. Ten specimens of *U. africana* were kept in a dish of aerated seawater as a control. The experiment was run at 20°C. Survival of the shrimps was checked at irregular intervals. The blood pO_2 of an additional 10 specimens of *U. africana* kept in water-saturated air was measured after 24 h exposure.

Oxygen consumption

Oxygen consumption of *U. africana* at controlled temperatures was measured in a flowing-water respirometer. A four-channel peristaltic pump transferred filtered seawater (34‰) from a 25 l glass reservoir to four respirometer chambers. Water leaving the chambers could be either diverted to waste or passed over an oxygen electrode (Radiometer). A bypass system was used to measure the pO_2 of water entering the chambers. Experimental animals were kept for 24 to 72 h in clean tanks containing aerated seawater. They were then put individually into a respirometer chamber and allowed to acclimiate for 2 h. During this period the flow was adjusted so that the pO_2 of effluent water from chambers was about 85% of that of influent water. Influent and effluent oxygen tensions, and water flow were measured for three 10-min periods for each animal. At the end of the experiment the animals were removed, their surface moisture was dried off with a paper towel, and they were weighed to the nearest 0.01 g. Consumption at 20 ± 0.5°C was determined on 60 shrimps in the range 0.2–3.5 g. Shrimp were collected in summer and the experimental temperature corresponded to the mean habitat temperature at that time (Hill and Allanson, 1971). Since activity affects oxygen consumption, measurements used in this paper are restricted to those taken when the shrimp exhibited slight activity—slow walking movements and slow pleopod beat. Vigorous activity involving rapid pleopod beat usually continued only for short periods.

Oxygen tension of blood

The rate at which the oxygen tension of blood dropped after exposure to low oxygen tension was determined on four batches each of 10 specimens of *U. africana*. The shrimps were kept for 24 h at a pO_2 of 140–148 mm Hg and a temperature of 20°C, in the dishes used for the anoxia tolerance experiments described above. The water was then replaced by water equilibrated to a pO_2 of 38 mm Hg and the tension maintained by vigorous bubbling with a gas mixture at the same tension. Blood was sampled from one batch after 1 h, a second after 3 h, a third after 13 h, and the fourth after 22 h. A control batch was kept in aerated seawater.

In a second experiment the relationship between blood oxygen tension (p_iO_2) and surrounding water tension (p_eO_2) was determined on four batches of 10 shrimp each. Each batch was kept at a different oxygen tension (112, 71, 38, or 19 mm Hg) for 6 h before blood samples were withdrawn. In all cases blood samples were extracted from the pericardial space by means of a syringe, injected into a Radi-

ometer BMS3 blood gas analyzer, and the pO_2 measured. The analyzer water bath was maintained at 20°C.

Results

Behavior at simulated low tide

About 1 hr after a simulated low tide began, *U. africana* in either glass burrows or mud burrows in an aquarium moved slowly up the burrow and approached the air-water interface. When its antennae reached the surface, the shrimp stopped, and in some cases moved down the burrow, before recommencing upward movement. Intermittent strong pleopod beat lowered the water level in the arm of the burrow containing the shrimp. The shrimp gradually moved higher in the burrow until its cephalothorax was out of the water and its abdomen was still submerged (Fig. 1). In this position the hindmost edge of the branchial chamber was underwater and the chamber remained full of water. The scaphognathites continued beating and a stream of water flowed from the anterior end of the gill chamber. This water ran down the ventral and ventrolateral surfaces of the shrimp and into the burrow water. At irregular intervals the animals retreated briefly beneath the surface. If an emerged shrimp was disturbed it retreated underwater but re-emerged within a few minutes. The mean pO_2 of water surrounding *U. africana* half emerged in this static system was 54.3 mm Hg (standard deviation, SD, 7.37, n = 10).

During simulated low tide, *U. capensis* gradually approached the surface, eventually emerging so that the water level was at the thorax-abdomen junction or even

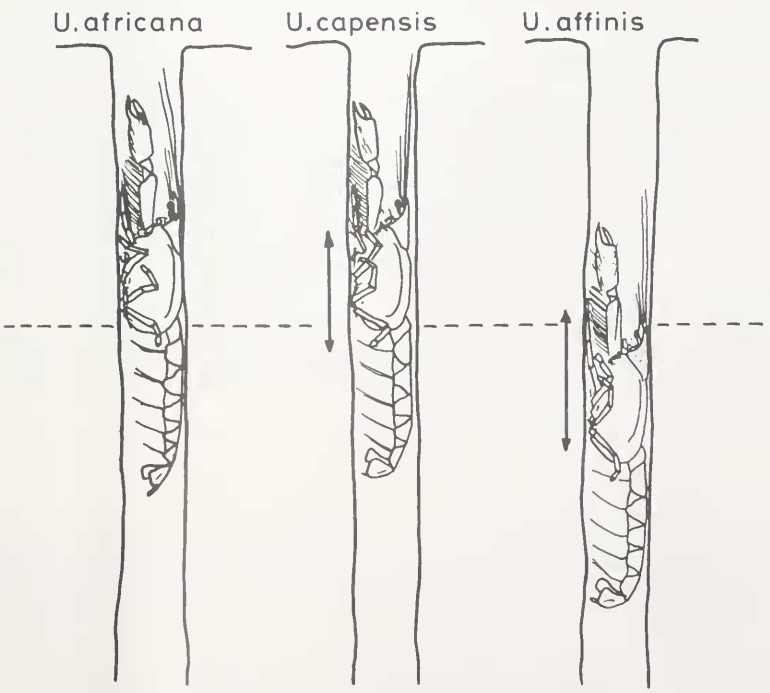


FIGURE 1. Positions assumed by three species of *Upogebia* at low tide. Dashed line indicates equilibrium level of burrow water. Vertical arrows indicate water movement induced by shrimp.

halfway down the abdomen. Strong pleopod beat then oscillated water in the burrow so that it washed up and down over the posterior end of the branchial chamber (Fig. 1). The caudal fan occasionally made a strong scooping movement which lifted water over the cephalothorax. The scaphognathite beat continued, maintaining a stream of water through the gill chamber.

U. affinis also slowly approached the surface, and made several rapid pleopod beats before reaching it. When these beats stopped, the caudal fan pushed water forward. The shrimp repeated this several times, occasionally walking upwards a short distance on the pleopod beat. When the shrimp reached a level at which the pleopod beat pulled the water level down to the tip of the rostrum, the pleopod beat increased, pulling the water further down. The animal gradually moved higher until its rostrum was approximately at the undisturbed water level. Pleopod beat now drew water down until its surface was approximately level with the junction of thorax and abdomen (Fig. 1). The water was held at this level by the pleopod beat and by a ventral flexing of the sixth abdominal segment, which caused the caudal fan to act as a barrier. After a few seconds to several minutes, the shrimp stopped its pleopod beat and relaxed its caudal fan, suddenly releasing the water. This caused the water level to rise rapidly up over the cephalothorax. The extent to which water was pulled down varied; the maximum was halfway down the abdomen. One specimen never exposed its body above water.

Thus, at simulated low tide, all three species took up a position in which the anterior half of the body was in air but in which the scaphognathites still pumped water through the branchial chamber. The shrimps appeared to avoid drying of the cephalothorax by periodic wetting, although by a different mechanism in each species.

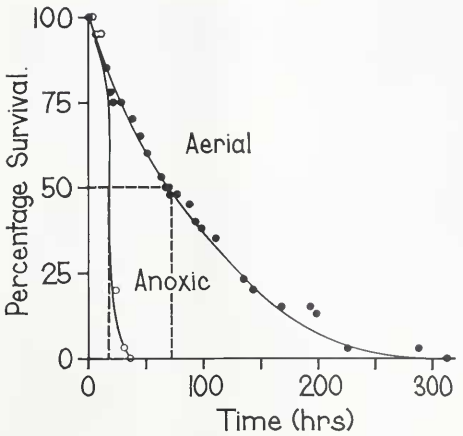
Effect of low oxygen tensions

U. africana exposed to low pO_2 in a glass burrow showed the same behavior as was observed under simulated low tide conditions. Various stages of the behavior pattern could be related to particular ranges of pO_2. Shrimp remained submerged when aerated water at a pO_2 of 145–150 mm Hg was passed through the burrow for up to 15 h. If the pO_2 was lowered below 36 Hg, activity decreased, the pleopod beat slowed, and the gills were frequently probed by the fifth pereiopods. The shrimps emerged when the pO_2 dropped below 11 mm Hg. When the pO_2 was raised above 25 mm Hg, they descended and remained submerged but were not very active. The pO_2 at which shrimp emerged in this flowing water system was lower than that required in the static system (see above). The difference may be due to a buildup of metabolites in the latter.

Tolerance to anoxia and survival in air

Because of the similar results of three replicate experiments on survival of *U. africana* in anoxic water, the results were grouped and an overall percentage survival was calculated (Fig. 2). Nearly all the shrimp survived anoxia for about 12 h, but died with longer exposure. Median survival time was 18 h. No shrimp died in the control experiment.

Fifty percent of specimens exposed to air died within 72 h (Fig. 2). At this time, 90% of control animals were still alive. The mean pO_2 of *U. africana* blood after 24 h in air was 37 mm Hg (standard error, SE, of mean 2.9).



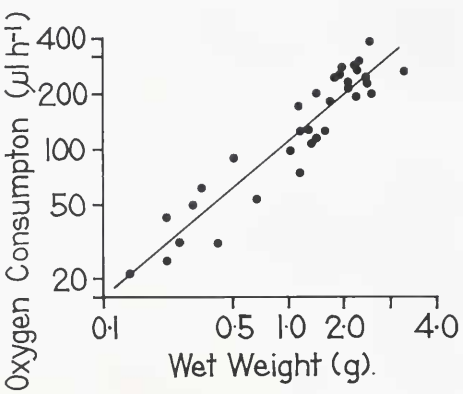


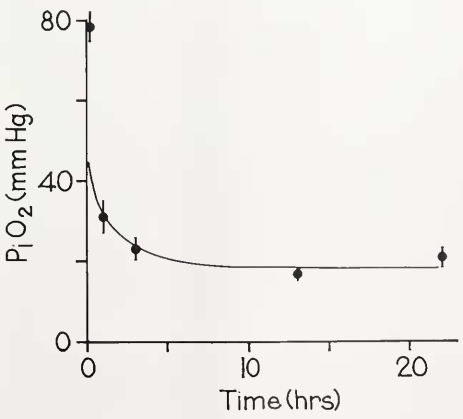
FIGURE 2. (left) Survival of *U. africana* specimens in anoxic (open circles) and aerial (solid circles) conditions. In each case 100% = 40 shrimps. Lines fitted by eye. Median time of survival in anoxia 18 h, in air 72 h.

FIGURE 3. (right) Oxygen consumption of *U. africana* specimens of various sizes at 20 ± 0.5°C. Line fitted by regression analysis (r^2 = 0.8823, n = 60).

Oxygen consumption and oxygen tension of the blood

The relationship between wet weight of *U. africana* and oxygen consumption ($\mu l \cdot h^{-1}$) at 20°C is shown in Figure 3, and is described by the equation log oxygen consumption ($\mu l \cdot h^{-1}$) = log 112.2 + 0.8446 log wet weight (g). The 95% confidence interval for b (slope) is 0.7321–0.9571.

The oxygen tension of *U. africana* blood declined rapidly when the shrimp were exposed to a pO_2 of 38 mm Hg (Fig. 4). A p_iO_2 of around 20 mm Hg was reached after 5 h and maintained for at least 22 h. In normoxic water (pO_2 140–150 mm



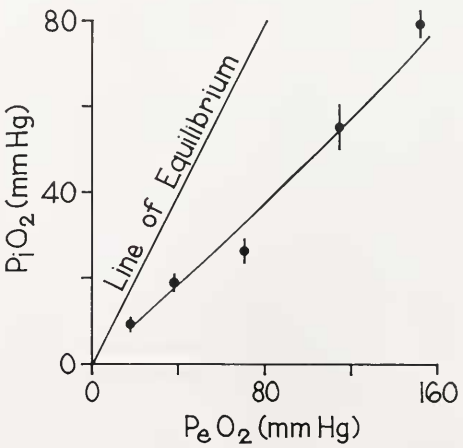


FIGURE 4. (left) Oxygen tension of the blood of *U. africana* specimens exposed to a pO_2 of 38 mm Hg (n = 10 at each point, vertical lines indicate 2 SE).

FIGURE 5. (right) Partial pressure of oxygen in the blood of *U. africana* specimens at various environmental oxygen tensions (n = 10 at each point, vertical lines indicate 2 SE).

Hg), *U. africana* had a p_iO_2 of around 80 mm Hg. In lower oxygen tensions the difference between p_iO_2 and p_eO_2 decreased but the blood oxygen tension was always approximately half that of the surrounding water (Fig. 5).

Discussion

Several measurements of oxygen consumption by thalassinids are now available. Thompson and Pritchard (1969) reported rates of 30 and 60 $\mu l \cdot g^{-1} \cdot h^{-1}$ at 10°C for 3–8 g specimens of *Callianassa californiensis* and *U. pugettensis*, respectively. Felder (1979) found similar rates (50–60 $\mu l \cdot g^{-1} \cdot h^{-1}$) for 3.5–5 g specimens of *C. jamaicense* at 25°C. Torres *et al.* (1977) reported a low rate of 14 $\mu l \cdot g^{-1} \cdot h^{-1}$ at 14°C for 10–15 g specimens of *C. californiensis*, and Miller et al. (1976) measured 30–40 $\mu l \cdot g^{-1} \cdot h^{-1}$ for 6–7 g specimens of the same species. Comparison of rates is difficult because size and temperature affect oxygen consumption. *U. africana* is a smaller species than the thalassinids used in the above studies. The average adult *U. africana* obtainable (3 g) used around 100 $\mu l \cdot g^{-1} \cdot h^{-1}$ at 20°C. This rate is comparable to those reported for similar sized thalassinids and confirm the general trend noted by previous authors that thalassinids have low metabolic rates as compared to other crustacea. Thompson and Pritchard (1969) and Felder (1979) considered that these low metabolic rates reflect an adaptation to a hypoxic habitat.

The size of *U. africana* burrows varies widely, but personal observations indicate that on average a 2 g shrimp occupies a burrow with a volume around 80 ml. At 20°C this volume of seawater contains about 400 μl O_2. A 2 g *U. africana* would consume approximately 200 μl $O_2 \cdot h^{-1}$. Thus the oxygen in the burrow water is insufficient to supply the occupant over a low tide. The blood pO_2 of *U. africana* drops rapidly when the shrimps are exposed to low oxygen (Fig. 4). According to Miller *et al.* (1976), *C. californiensis* blood has a low oxygen content. Thus twice each day at low tide, thalassinids have to use alternative mechanisms to cope with hypoxic conditions. The short survival of *U. africana* in anoxic conditions (Fig. 2) indicates that, in this species, anaerobic metabolism is not well developed. All species of *Upogebia* so far tested appear to have a low tolerance to hypoxia. Thompson and Pritchard (1969) showed that *U. pugettensis* was less tolerant of anoxic stress than was *C. californiensis*. Part of the mechanism of this difference was shown by Miller *et al.* (1977) to be due to physiological properties of the blood, in that hemocyanin of *C. californiensis* has a higher affinity for oxygen (p_{50} 4 mm Hg) than that of *U. pugettensis* (p_{50} 11.5 mm Hg).

When exposed to hypoxic conditions in a simulated burrow, specimens of all three species of *Upogebia* tested in the present study showed a characteristic behavior that involved moving to the air-water interface. Farley and Case (1968) reported that *C. californiensis* appears to move towards the openings of its burrows at low tide. Felder (1979) found specimens of *C. jamaicense* in the substrate well above the water table. He suggested that since *C. jamaicense* can survive and respire in water-saturated air, occupancy of exposed upper portions of burrows seems a plausible alternative to long-term anoxia.

U. africana appears to have some ability to respire in air, since it survived better out of water than in anoxic conditions. Nevertheless, the shrimp's oxygen uptake in air was not highly efficient, since in air its p_iO_2 (37 mm Hg) was half that in water (78 mm Hg). Mortality in air was probably not due to lack of oxygen but to a multiplicity of factors, including a buildup of metabolic products. The behavior of the three species of *Upogebia* at low tide did not involve the shrimps leaving the water completely. They took up a position in which water could be pumped through

the gill chamber despite the fact that the cephalothorax was exposed. This water, being at the air-water interface, could be expected to have a higher oxygen content than that lower down in the burrow. Water trickling back down over the ventral surface of the shrimp after leaving the gill chamber may be partly reoxygenated. A similar mechanism has been described in the grapsid crab *Sesarma catenata* by Alexander and Ewer (1969). In this crab water can be stored in the gill chamber, whereas in *Upogebia*, continued irrigation of the gills relies upon the shrimp's maintaining contact with burrow water.

Callianassa species live in temporary burrows in permeable sandy substrates. They are continually exposed to low levels of oxygen and require a well developed tolerance to hypoxia. A similar high resistance to anoxic stress is not necessary in *Upogebia* because the shrimps live in a burrow which is sealed from interstitial water (Thompson and Pritchard, 1969) and have a specialized respiratory behavior at low tide.

Acknowledgments

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